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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/897,988	07/05/2001	Yuta Nakai	210669US0	1677

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AJINOMOTO CORPORATE SERVICES, LLC
INTELLECTUAL PROPERTY DEPARTMENT
1120 CONNECTICUT AVE., N.W.
WASHINGTON, DC 20036

EXAMINER	
MARVICH, MARIA	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 08/09/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/897,988	NAKAI ET AL.	
	Examiner	Art Unit	
	Maria B Marvich, PhD	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 May 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8, 10* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8/11/03*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to an amendment filed 8/11/03 and a supplemental amendment filed 5/17/04. The supplemental amendment was filed to provide portions of the amendment inadvertently omitted from the amendment filed 8/11/03. Claim 1 has been amended. Claim 9 has been cancelled. Claim 10 has been added. Claims 1-8 and 10 are pending in this application.

Response to Amendment

Any rejection of record in the previous action not addressed in this office action is withdrawn. The new grounds of rejection herein were necessitated by amendment and, therefore, this action is final.

Information Disclosure Statement

An IDS filed 8/11/03 has been identified and the documents considered. The signed and initialed PTO Form 1449 has been mailed with this action.

Specification

Upon reconsideration, the substitute specification filed 1/9/03 has not been entered as amendments to the specification do not put the specification in proper idiomatic English. There are multiple passage of improper idiomatic English. The following examples are **representative** of the improper idiomatic English. On page 10, line 2 "This procedure will be explained hereafter by exemplifying cyo operon (*cyoABCDE*) coding for a cytochrome bo type oxidase as a

gene of respiratory chain enzyme of high energy efficiency." On page 10, line 7 "It is also possible to use a gene of bacterium belonging to the genus *Escherichia*, or a gene derived from other organisms such as corneyform bacteria as the cyo operon." On page 11, line 21, "The amplification of cytochrome bo type oxidase activity can also be attained by allowing existence of multiple copies of the cyo operon on chromosomal DNA of host". Therefore, a substitute specification in proper idiomatic English and in compliance with 37 CFR 1.52(a) and (b) is required (excluding claims). A statement that it contains no new matter must accompany the substitute specification filed.

The disclosure is also objected to because of the following informalities: on page 11, line 8 "methods" should be singular and on page 19, line 1, "cytochrome" is misspelled.

Appropriate correction is required.

Claim Objections

Claim 7 is objected to because of the following informalities: claim 7 is missing a word between "The" and "according". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 10 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **This is a new rejection necessitated by applicant's amendment.**

Claim 10 is vague and indefinite in that the metes and bounds of "at least one bacterium" are unclear. It is unclear if "at least one bacterium" means that at least one type of bacterium is in a mixed culture or that the microorganism is more than one microorganism of the same type.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-8 and 10 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of producing a target substance utilizing microorganisms with an enhanced SoxM type oxidase or NDH-I activity and deficient cytochrome bo type oxidase or NDHII activity, it does not provide an enabling disclosure for said method using any microorganism with any enzyme constituting the respiratory chain pathway with high energy that is enhanced and/or with low energy efficiency that is deficient. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. **This rejection is maintained for reasons of record in the office action mailed 4/9/03 and is extended to newly added claim 10.**

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat.

App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

1) **Nature of the art**. The instant application recites a method for the production of target substances following strain improvement. These improved microorganisms are engineered to contain altered genes of the respiratory chain pathway in which enzymes of high efficiency are enhanced by amplification of native genes, mutagenesis of native promoters or the introduction of regulatory genes. Alternatively or in concert with the aforementioned mutations, enzymes of low efficiency of the respiratory pathway are made deficient by mutation or deletion. The strain is then used for the production of any target substance.

2) Scope of the invention. The invention recites a method for producing target substances through genetic manipulation of bacterial strains. The scope is broad in that the claims recite use of any microorganism that is a mutant strain or genetic recombination strain with an enhanced high-energy respiratory chain and/or deficient low energy respiratory chain. The invention relies primarily on the fields of microbiology and recombinant technology.

3) Number of working examples and guidance. It is disclosed that there are two proton excitability or energy pathways in microbe aerobic respiration, a high energy and a low energy pathway. The instant disclosure teaches the development of *E. coli* strains with specific alterations in the expression levels of cytochrome bo type oxidases (high energy respiratory enzymes and NDH-II (low energy respiratory pathway enzymes). The specification teaches broadly that means of over expression can be achieved through gene amplification, promoter enhancement and mutagenesis while a deficiency is created by gene reduction or elimination through homologous recombination and mutation. Specifically, disclosed is an expression

system with amplified bo type oxidases (overproducing cyo strains) or deletion of the gene encoding NDH-II. The effect of these strains in the production of L-lysine, l-phenylalanine and L-threonine is also provided as to strains, growth conditions, and assays for production.

4) **State of Art** The state of the art at the time of invention included knowledge of respiration systems in *E.coli* rooted in many years of analysis. The analysis of energetic efficiency and the detailed analysis of respiratory pathways are still under study. We are taught in the specification that the enzymes cytochrome bd type oxidase and NDH-II are low efficiency enzymes while cytochrome bo type oxidase and NDH-I are high efficiency enzymes. In *E.coli,* cytochrome bo is considered a SoxM type oxidase. The prior art does not identify any other genes for which the state of their energy efficiency has been determined.

5) **Unpredictability of the art**. The guidance provided by the applicants does not teach how to predict what other strains of bacteria with altered high and low efficiency enzymes would be suitable.

As Bailey (Science, June 1991) teaches, metabolic engineering "enables construction of metabolic configurations with novel and often beneficial characteristics" but "at present, metabolic engineering is more a collection of examples than a codified science" (page 1668, column 1, paragraph 2 and 3). Furthermore, he teaches "Many studies have shown the feasibility of metabolic engineering methods without achieving the yields, rates or titers (final concentrations) required for practical processes." (page 1668, column 1, paragraph 3). The obstacles Bailey details are that the cell has complex cellular responses to genetic perturbations that complicate predictive design. The complex cellular responses include an inability to anticipate further reactions which leads to iterative cycles of genetic modifications, an inability

to predict metabolic consequences following the transfer of heterologous genes into the cell as well as rearrangements and deletions of chromosomal and plasmid DNA.

More recently, Parekh et al. (Appl Microbiol Biotechnol., 2000) teach that "...heterologous protein expression in bacterial or fungal systems offers a significantly complex, metabolic network. The rate-limiting enzymatic reactions or flux nodes are unknown in most if not all pathways and probably change with each new culture. Thus with limited knowledge of the physiology and genetics associated with the production of each molecule of interest one is often led to an empirical approach to strain improvement. (page 288, column 1, paragraph 1)." Further problems are encountered with the need to scale-up processes such as false positives are encountered and an inability to maintain the same physical environment (page 299, column 1, paragraph 2-3).

Specifically, for the system designed in the instant application, the art of discernment of energetic efficiency is a contested topic. Identifying genes other than NDH-I, NDH-II, cytochrome bd and cytochrome bo is not guaranteed as the means to determine the efficiency of energy of the proteins these genes encode is an unpredictable art. As taught by Neijssel and de Mattos (Molecular Microbiology (1994) 13(2), 179-182), " the efficiency of energy conservation by bacterial respiratory chains using intact cells could not (and still cannot) be determined as simply as in mitochondria and cell-free preparations yielded unreasonably low P/O ratios" (page 180, column 1, paragraph 1). They further teach "it can be shown that there are many energy-spilling reactions in the cell whose physiological functions are sometimes unclear (e.g. futile cycles), but whose activity in vivo may well vary with growth rate. It is extremely difficult if not impossible to determine the activities of these reactions in growing

cultures, and this implies that one cannot derive a reliable estimate of the efficiency of the respiratory chain from measurements of growth yields" page 180, column 2, paragraph 2).

6) **Summary**. The level of skill in the art covering this invention was high at the time of invention. However, given the unpredictability of the art, the poorly developed state of the art and the lack of guidance presented by applicant, the skilled artisan would have needed to conduct undue and excessive experimentation to practice the claimed invention.

In view of predictability of the art to which the invention pertains and the lack of: undue experimentation would be required to practice the claimed methods with reasonable expectation of success, absent a specific and detailed description in the specification. Given the above analysis of the factors which the courts have determined are critical in determining whether a claimed invention is enabled, it must be concluded that the skilled artisan would have had to have conducted undue unpredictable experimentation in order to practice the claimed invention.

Response to Amendment

Applicants traverse the claim rejections under 35 U.S.C. 112, first paragraph on pages 4-8 of the amendment filed 8/11/03. Essentially, the applicants argue the following. 1) Microorganisms other than *Escherichia* and *Corneyform* have respiratory chains involved in a respiratory pathway. To this end, applicants have submitted six references demonstrating the occurrence of these enzymes in *Bacillus subtilis, Paracoccus denitrificans, Synechocystis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Azotobacter vinelandii* and *Bacillus stearothermophilus*. 2) Applicants provide documentation of the relatedness between SoxM type oxidases and conclude that it was well known by a person of skill in the art that such enzymes are similar to those of *Escherichia coli*. 3) Given the similarity between the enzymes, one of

skill could isolate the enzymes and using the means of enhancing or reducing provided in the specification perform the instant invention. This coupled with the high level of skill in the art is said to "clearly outweigh and overcome any unpredictability that may have been present long ago". 4) Applicants argue that most of the disclosures are old except Parekh et al. However, the full disclosure of Parekh et al teaches that the level of unpredictability is low. 5) Finally, applicants argue that as the claims do not recite scaling up, disclosures related to scaling up should not be relied upon in rejecting the instant claims.

Applicant's arguments filed 8/11/03 have been fully considered but they are not persuasive. The specification has demonstrated that *E. coli* strains containing amplified bo type oxidases (overproducing cyo strains) or deletion of the gene encoding NDH-II perform as strains with high-energy efficient respiratory chain pathways and or deficient low energy efficiency respiratory chain pathways. It is unpredictable that applicant's invention can be performed with any microorganism with any enzyme constituting the respiratory chain pathway with high energy that is enhanced and/or low energy efficiency that is deficient. While applicants have demonstrated that cytochrome bd and SoxM type oxidases have been identified in a variety of microorganisms, the relationship of the depicted enzymes to the instantly disclosed enzymes are unclear. For example, applicants have indicated that cytochrome bo is an enzyme of the high-energy efficiency pathway. However, it is unclear if all SoxM oxidases are part of the high-energy efficiency pathway and furthermore, the relationship of the SoxM enzymes to cytochrome bo. Applicants have stated that a person of skill in the art would conclude that it was well known by a person of skill in the art that such enzymes are similar to those of *Escherichia coli*. However, the basis of the statement is unclear. For example, included in the phylogenetic

tree of Sakamoto et al (figure 5), cyo B of *E. coli* is included. This is but one component of the high-energy pathway described in the instant specification and its value alone in the instant invention is unknown. The prior art does not identify any other genes for which the state of their energy efficiency has been determined. Therefore, it is unpredictable that the cloning and mutation of any of the presently disclosed SoxM oxidases and cytochrome bd oxidases from any strain will perform the same function as the disclosed strain. The specification as well as the prior art, solely teaches that the cytochrome bd oxidase and NDH-II are low efficiency enzymes while cytochrome bo type oxidase and NDH-I are high efficiency enzymes.

While it is clear that classical strain improvement has a long history of success, this does not predict that for all strain improvement there will be success. Parekh teaches that "overproduction of primary or secondary metabolites is a complex process and successful development of improved strains requires a knowledge of physiology, pathway regulation and control and the design of creative screening procedures" (page 287, column 1). In the instant application, there is a lack of knowledge about the energy efficiency of enzymes other than those disclosed for *E.coli.* Therefore, there is a lack of knowledge of the physiology, pathway regulation and control and no discussion of "creative screening procedures" for the required enzymes.

Finally, the office has not relied solely on the teachings of Parekh et al that demonstrate problems encountered with the need to scale-up processes to illustrate the unpredictability of the instant invention. Other factors detailed in the rejection above have contributed to the conclusion that it is unpredictable to practice the instant invention with any microorganism with alterations in any high or low energy efficiency pathway. However, it is readily known to one of

skill in the art that target production is most useful in industrial production of target substances and in such cases scaling up is a necessary component. Even though Parekh discusses issues associated with scaling up, it is not clear why this disclosures should not be relied upon in rejecting the instant claims as it also discusses the obstacles and potentials of microbial strain improvement. For example, applicants have relied upon Parekh et al as indication that the instant invention is predictable.

Claims 1-8 and 10 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This rejection is maintained for reasons of record in the office action in the office action mailed 4/9/03 and is extended to newly added claim 10.**

Applicants claim a genus of microorganisms constructed from a parent strain that has a respiratory pathway of high energy efficiency and a respiratory pathway of low energy efficiency and that is a mutant or genetic recombinant having either or both an enhanced respiratory chain pathway of high energy efficiency and a deficient respiratory chain pathway of low energy efficiency.

The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed

correlations between function and structure, or by a combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus.

The instant invention recites as an essential element for the production of target substance, improved microorganisms obtained from parent strains that are engineered to contain altered high and low energy efficiency genes of the respiratory chain pathway. The structure of a species is disclosed as the specification discloses that the *E. coli* strains W3110 and VKPM B-3996 are parental strains for the construction of the overproducing cyo strains and deficient NDHII strains. The specification does not disclose relevant identifying characteristics of the nature of the mutation or genetic engineering sufficient to describe the steps to attain the mutant strains with the derived high energy or low energy efficiencies in such full, clear, concise, and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention. Nor does the specification disclose a correlation between the mutations or genetic changes in the exemplified cell, *E. coli*, and those required in other organisms to produce the necessary changes in the respiratory pathway for use in the claimed invention. For inventions in an unpredictable art, adequate written description of a genus cannot usually be achieved by disclosing only one species within the genus. Furthermore, the specification and the prior art has not established a strong correlation between the structure of mutated or genetically altered enzymes and their function in altering respiratory pathways in the organism and one skilled in the art cannot predict with a reasonable degree of confidence the structure of the claimed invention from the recitation of its function. Therefore, the skilled artisan cannot conclude that the applicant was in possession of the claimed invention.

Response to Arguments

To be completely responsive to the Office action mailed 4/9/03, applicants should have provided a specific response to the rejection of claims 1-10 under 35 USC 112, first paragraph for lack of written description. The amendment appears to be a bona fide attempt to respond completely to the office action mailed 4/9/03 as it appears that applicants intended that arguments directed against the rejection of claims 1-10 under 35 USC 112, first paragraph for lack of enablement are intended to be the same. The Under 35 USC 1.135c), " When a bona fide attempt to reply includes an omission that does not preclude action on the merits of the application (e.g., a reply fails to address a rejection or objection), the examiner may waive the deficiency in the reply and act on the application. The examiner may repeat and make final the rejection, objection, or requirement that was the subject of the omission." Due to the many delays in prosecution of this case, this omission is waived.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B Marvich, PhD whose telephone number is (703) 605-1207. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel, PhD can be reached on (703) 305-1998. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 305-4242 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Maria B Marvich, PhD
Examiner
Art Unit 1636

GERRY LEFFERS
PRIMARY EXAMINER

August 4, 2004